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Evaluation of alterations in physical, hematobiochemical and ultrasonography images following chemosterilization using calcium chloride intratesticular injection in Wistar rats

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Abstract

Street animal overpopulation poses serious public health and economic concerns due to their role as reservoirs and vectors of infectious diseases. Chemical sterilization offers a practical, economical, and less labor-intensive alternative to surgical and hormonal methods for mass population control. This study evaluated the effects of intratesticular calcium chloride (CaCl₂) injection on physical, hematobiochemical, and ultrasonographic parameters in Wistar rats and assessed the influence of meloxicam on treatment response. Eighteen rats were randomly divided into three groups: control (normal saline), T₁ (20% CaCl₂), and T₂ (20% CaCl₂ + meloxicam). Clinical signs, body weight, hematological indices, serum biochemistry, lipid peroxidation, and ultrasonographic changes were recorded over 30 days. Mild reduction in activity was observed in treated groups during the initial two days, with no mortality reported. Transient testicular swelling persisted for 48 hours post-injection and resolved by day five. Hematobiochemical findings indicated inflammatory and oxidative stress responses, while ultrasonography revealed progressive increases in echogenicity suggestive of degeneration and fibrosis of testicular tissue. Overall, calcium chloride intratesticular injection proved to be an effective, minimally invasive, and economical chemosterilization technique with limited systemic effects, supporting its potential application for large-scale animal population control programs.

Keywords: Chemosterilization, calcium chloride, intratesticular injection, Wistar rats, ultrasonography

Introduction

Street animal overpopulation is a significant issue in many places all over the world because they can serve as reservoirs or vectors for diseases that are deadly to humans as well as economically valuable domestic species. This causes the public health issues, even the vaccination against these diseases to stray animals is practically not possible. So to combat it there should be control on the increasing population which can be achieved through sterilization of animals.

Sterilization can be done by Surgical, Chemical and hormonal methods. Surgical, hormonal or by Vaccination methods are costly and time-consuming. Chemical sterilization can be the alternative to both Surgical and hormonal sterilization being less expensive and less labor intensive, which allows mass-scale sterilization quickly and safely (Taylor *et al.*, 2017).

Various chemical agents such as ferric chloride (Kar *et al.*, 1965), danazol (Dixit *et al.*, 1975), BCG (Das *et al.*, 1982), zinc tannate (Ansari *et al.*, 2017), zinc gluconate (Oliveira *et al.*, 2007), zinc arginine (Chaudhary *et al.*, 2018), glycerol (Weinbauer *et al.*, 1985; Immegart and Threlfall, 2000; Gunay *et al.*, 2004), glucose, NaCl (Heath and Arowolo, 1987; Russell *et al.*, 1987) dibromochloropropane (Shemi *et al.*, 1988), lactic acid (Fordyce *et al.*, 1989), zinc arginine (Fahim *et al.*, 1993), sodium fluoride (Sprando *et al.*, 1996), formalin (Bakir *et al.*, 2002), calcium chloride (Jana *et al.*, 2002; Jana and Samanta, 2011; Hami *et al.*, 2020), ethanol (Canpolat *et al.*, 2006a), potassium permanganate plus glacial acetic (Giri *et al.*, 2002), mannitol (Maadi *et al.*, 2021) and cetrime (Fesseha and Negash, 2020) [2, 3, 4, 8, 11, 15, 16, 18, 19, 20, 21, 22, 24, 28, 35, 43, 44, 50, 52, 55, 57, 59].

And in ruminant animals, chemicals like lactic acid (Hill *et al.*, 1985), tannic acid and zinc sulphate (Feher *et al.*, 1985), alpha-hydroxy propionic acid (Cohen *et al.*, 1990), formalin (Ijaz *et al.*, 2000), Ethanol (Canpolat *et al.*, 2006b) and castrate-Quin (Soerensen *et al.*, 2001) are been used till date for the chemical sterilization [5, 10, 17, 25, 27, 36].

Calcium chloride (CaCl₂) is one of the most promising chemical agents, calcium chloride, has been used to chemically castrate a variety of species (Koger, 1978) [37]. Following intratesticular injection, calcium chloride induces necrosis, fibrosis, and degeneration of seminiferous tubules and Leydig cells, which, in a dose-dependent manner, reduces the production of spermatozoa, testosterone, and sperm counts. It results in both local and systemic reactions, including scrotal ulcers, dermatitis, abscesses, and fibrosis in the injected area, as well as peripheral edema, nausea, vomiting, diarrhoea, lethargy, and leukocytosis, which is followed by intratesticular injection (Canpolat *et al.*, 2006b, Leoci, 2012, Kutzler and Wood, 2006) [5, 38, 40].

After intratesticular injection of CaCl₂, signs of pain, pyrexia and even severe inflammation were observed (Jana and Samantha, 2006). Many a time anti-inflammatory drugs are used to reduce pain and inflammation. So, it is possible that anti-inflammatory treatment disturbs the desired action as a sterilizer [29].

Materials and Methods

Ethical approval: This experimental protocol (VETCOLL/IAEC/2022/19/ PROTOCOL-04) was approved by the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary Science and Animal Husbandry, Kamdhenu University (KU), Sardarkrushinagar, Gujarat, India.

Procurement of Rats: Total 18 Wistar rats were procured from the Laboratory Animal Facility of Torrent Pharmaceuticals, Gandhinagar, and Gujarat, India. All the rats were acclimatized for 7 days before enrolling in the studies. Animal management and treatment procedures compiled with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India. Rats were kept in polypropylene cages at the Laboratory Animal Facility in an environmentally controlled room with 22±3 °C temperature and 30-70% humidity. Light/dark cycles of 12/12 hours were given. Standard pellet food and Water were provided *ad libidum*.

Experimental design: Rats were divided in three groups, each comprising of 6 rats. The group were named as control group (Normal saline), treatment group T₁ (CaCl₂) and treatment group T₂ (CaCl₂ + Meloxicam). Meloxicam at the dose rate of 2 mg/kg body weight, was given by subcutaneous route.

Intratesticular injections: After applying local anesthetic intratesticular injection of either normal saline or 20% CaCl₂ was given with the use of insulin syringes at the dose rate of 0.1ml/100gram body weight as described by Jana *et al.*, 2002.

Clinical observations: All animals were observed for morbidity and mortality twice daily throughout the study. During the acclimatization period, the clinical observation

was recorded once daily. Clinical observations like rectal temperature and behavioral observations like activeness were recorded twice for 5 days. Following intratesticular injection presence of swelling was checked.

Body Weight: The body weight of all rats was recorded on day one of initiation of dosing and thereafter on days 5, 10, 20 and 30.

Blood Collection: Blood was collected on 0, 10th and 30th day of the study after anesthetizing with isoflurane. Blood was collected from retro-orbital plexus as described by Parasuraman *et al.* (2010) [47] with the help of a capillary tube in the sterilized vial containing 4.0 per cent Potassium Ethylene Diamine Tetra Acetic Acid (K3 EDTA) for the estimation of hematological parameters and in serum clot activator vial for biochemical estimates

Hematology

1. Total Erythrocyte Count (TEC) (106/μL)
2. Haemoglobin (Hb) (g/dL)
3. Packed Cell Volume (PCV) or Haematocrit (HCT) (%)
4. Platelets (PLT) (103/μL)
5. Total Leukocytes Count (TLC) (103/μL)
6. Differential Leukocytes Count (DLC)
7. Lymphocytes (103/μL)
8. Monocytes (103/μL)
9. Neutrophils (103/μL)
10. Eosinophils (103/μL)
11. Basophils (103/μL)

Aspartate aminotransferase (AST) (U/L)

Its estimation was carried out by the colorimetric method devised by DeVries *et al.*, 1976 using standard procedure and a diagnostic kit (Agappe, India). The working reagent was made by mixing 4 volumes of Reagent 1 (R1) with 1 volume of Reagent 2 (R2). For absorbance of blank only 500 μL working solution's OD was taken and for sample absorbance 500 μL and 50 μL was mixed and incubated for 1 minute, then 490 μL mixture aspired and change in absorbance per minute during 3 minutes was noted at 340 nm wavelength on Microlab RX-50V semiautomatic biochemical analyzer (Fig.3.8).

Lipid peroxidation (LPO)

Oxidative stress in serum was determined in terms of malondialdehyde (MDA) by using the method of Buege and Aust (1978).

Ultrasonographic Observation

Testis of rats was scanned with a frequency of 7.5 MHz Micro convex probe and Sonosite M-Turbo Fujifilm Ultrasonographic device. Ultrasonographic picture of testis were observed on 0, 5, 10, 20 and 30 days. Echogenicity was graded based on low brightness 1, medium brightness 2, high brightness 3 and very high brightness 4 (Hami *et al.*, 2020) [22].

Statistical Analysis

The data pertaining to Haematological, Biochemical, Rectal temperature and Body weights were compiled as mean±S.E. and analyzed by analysis of variance method, ANOVA in SPSS 20.0 software and significant differences among the treatments were determined using DMRT.

Results: Animals of the group T₁ and T₂ showed a slight decrease in their activeness for the first two days. But the rats of the control group had not expressed any change in their active behavior. Further, no mortality was observed during the whole study of 30 days in any of the groups.

Swelling of Testis: The presence of swelling in the testis after the intratesticular inject groups group T₁ and T₂ group

was noticed for 48 hours, (Fig. 4.1 and 4.2) which diminished gradually and disappeared totally after 5 days. While no such pronounced swelling was evident in any of the testis of control group rats (Fig.4.3). There was no difference in the swelling of the testis between the T₁ and T₂ groups. There was no pain on palpation, but there was a slight increase in the firmness in the testis following intratesticular injection for up to 24 hours.

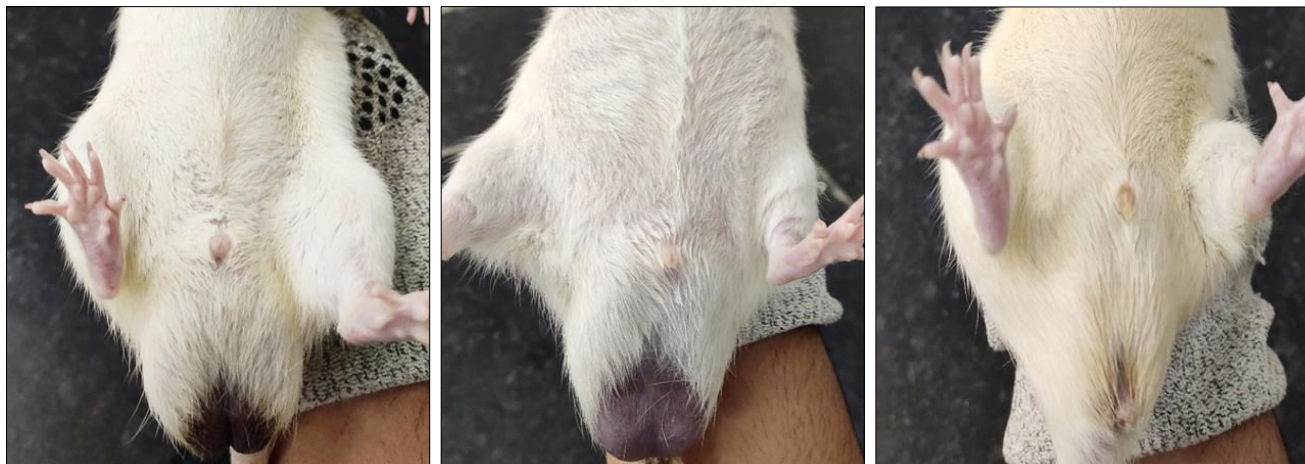


Fig 4.1: Group T₁. Presence of scrotal swelling, **Fig 4.2:** Group T₂. Presence of scrotal swelling, **Fig 4.3:** Control group. Scrotum showing no change

Rectal Temperature: There was no significant difference in the rectal temperature on day 0 among the different groups. However, on day 1 highly significant elevation of rectal temperature was observed in the T₁ and T₂ groups as compared to the control group. But there was no difference between the T₁ and T₂ groups. Further, on days 2 and 3 the highly significant rise in rectal temperature was noticed only in the group T₁. And there was no difference in rectal

temperature between the control and T₂ groups. During the period of observation which is from day 0 to day 5, no significant change was observed in the control group. In the T₁ group, the temperature remained elevated up to day 3 which comes equal to day 0 on day 4. But in the T₂ group, the elevated temperature was seen only on day 1, which reduced on day 2 and come to the temperature recorded on day 0 from day 4 onwards.

Table 4.1: Effect of intratesticular injection of calcium chloride on rectal temperature (°F) (Mean ±SE) on days 0, 1, 2, 3, 4 and 5 in male rats

Group/Period	Control group (n=6)	Treatment group T ₁ (n=6)	Treatment group T ₂ (n=6)	P value
Day 0	98.9±0.3	98.7±0.4 _B	99.5±0.4 _{AB}	0.387
Day 1	98.6±0.2 ^b	100.1±0.3 ^a _A	99.9±0.4 ^a _A	0.009**
Day 2	98.2±0.6 ^b	100.1±0.3 ^a _A	98.6±0.3 ^b _C	0.001**
Day 3	98.6±0.2 ^b	100.2±0.4 ^a _A	98.5±0.2 ^b _C	0.001**
Day 4	98.2±0.1	98.9±0.4 _B	99.0±0.1 _{BC}	0.125
Day 5	98.4±0.2	98.6±0.2 _B	98.7±0.2 _{BC}	0.491
P value	0.112	0.003**	0.004**	

Body Weight

Body weight is a frequent but important indicator. A drug or treatment's impact might be seen in the body weight change. The mean body weight in grams of rats of control, T₁ and T₂ groups taken on days 0, 5, 10, 20 and 30 is mentioned in Table 4.2 and Fig. 4.5. The mean body weight of rats of

group T₁ and T₂ showed no significant difference with the control group on day 0, 5, 10, 20 and 30, even no significant difference was seen between group T₁ and T₂. While looking at the period no significant difference between body weight Day 0, 5, 10, 20, and 30 in control and groups T₁ and T₂ was recorded.

Table 4.2: Effect of intratesticular injection of calcium chloride on body weight (gm) (Mean ±SE) on days 0, 5, 10, 20 and 30 in male rats

Group Period	Control group (n=6)	Treatment group T ₁ (n=6)	Treatment group T ₂ (n=6)	P value
0 day	499.83±13.35	507.33±24.58	502.67±24.64	0.970
5 day	490.00±13.34	466.83±26.11	483.00±24.68	0.754
10 day	506.83±15.71	465.00±25.01	464.83±22.81	0.312
20 day	506.33±17.92	473.33±23.44	462.50±24.92	0.375
30 day	513.50±17.98	478.83±23.65	481.50±27.22	0.518
P value	0.865	0.745	0.789	

On day 0, the echogenicity of testis noticed was hyperechogenic (grade 3, Fig. 4.11) with few anechoic areas in all rats of three groups. On day 5, the echogenicity of control group rats was showing anechoic area in the centre surrounded by hyperechoic spots (nearly grade 3, Fig. 4.12). However, in T₁ and T₂ group's rats the noticeable area with hyperechogenicity (grade 4, Fig. 4.13 and Fig. 4.14) was evident. On day 10 echogenicity of testis remained same in

control, T₁ and T₂ groups (Fig. 4.15, Fig. 4.16 and Fig. 4.17). On day 20, echogenicity in control group remained unaltered (Fig. 4.18) but in T₁ and T₂ group the anechoic area (grade 2, Fig. 4.19 and Fig. 4.20) were noticed. And on day 30 echogenicity of testis in control group rats remained same (Fig. 4.21) as in T₁ and T₂ groups complete anechoic testis were observed (grade 1, Fig. 4.22 and Fig. 4.23).

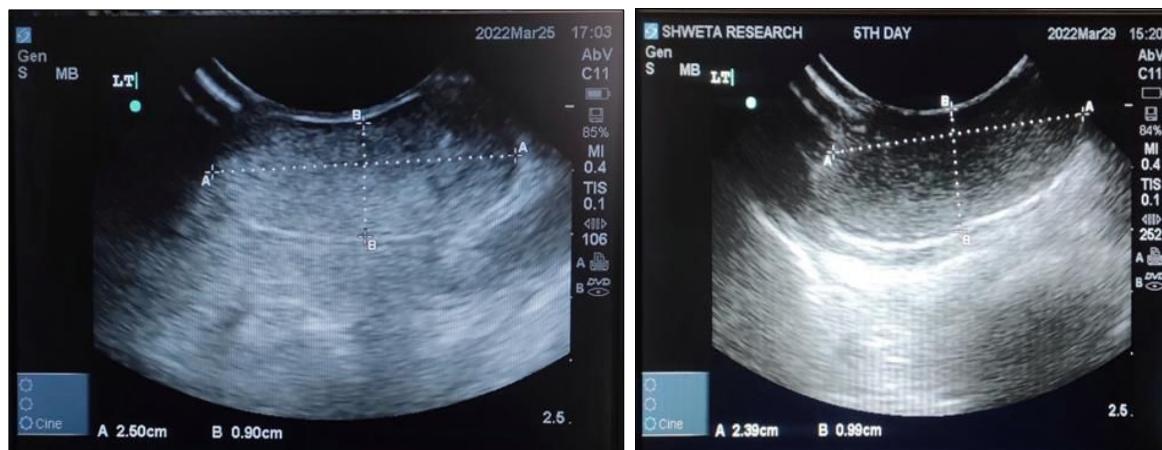


Fig 4.11: Echogenicity of testicles of all rats on day 0 (Grade 3), **Fig 4.12:** Control group. Echogenicity of testicles on day 5 (Grade 3)

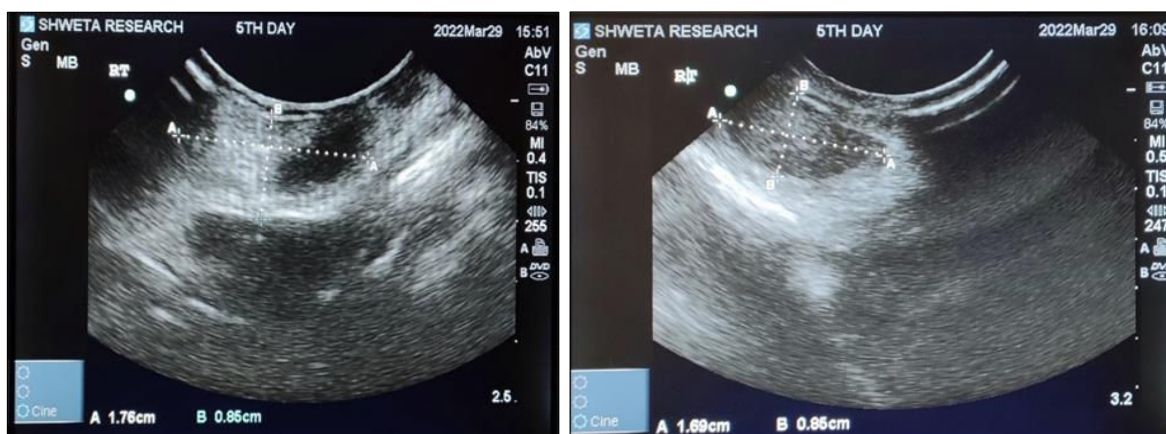


Fig 4.13: Group T₁. Echogenicity of testicles on day 5 (Grade 4), **Fig 4.14:** Group T₂. Echogenicity of testicles on day 5 (Grade 4)

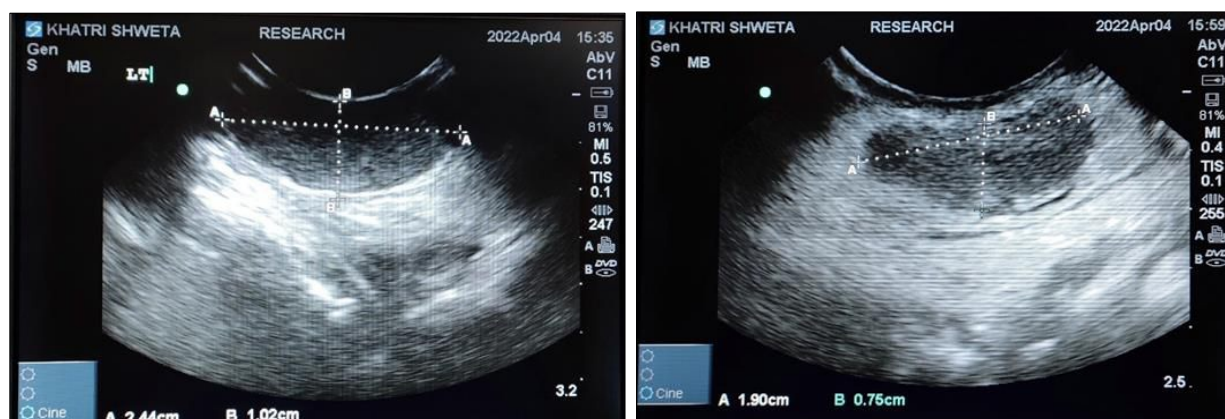


Fig 4.15: Control group. Echogenicity of testicles on day 10 (Grade 3), **Fig 4.16:** Group T₁. Echogenicity of testicles on day 10 (Grade 3)

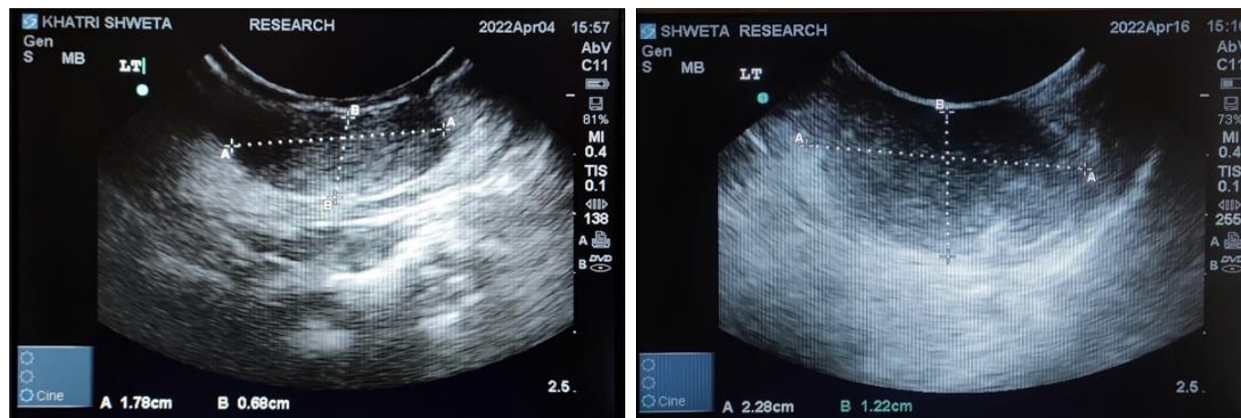


Fig 4.17: Group T₂. Echogenicity of testicles on day 10 (Grade 3), **Fig 4.18:** Control group. Echogenicity of testicles on day 20 (Grade 3)



Fig 4.19: Group T₁. Echogenicity of testicles on day 20 (Grade 2), **Fig 4.20:** Group T₂. Echogenicity of testicles on day 20 (Grade 2)

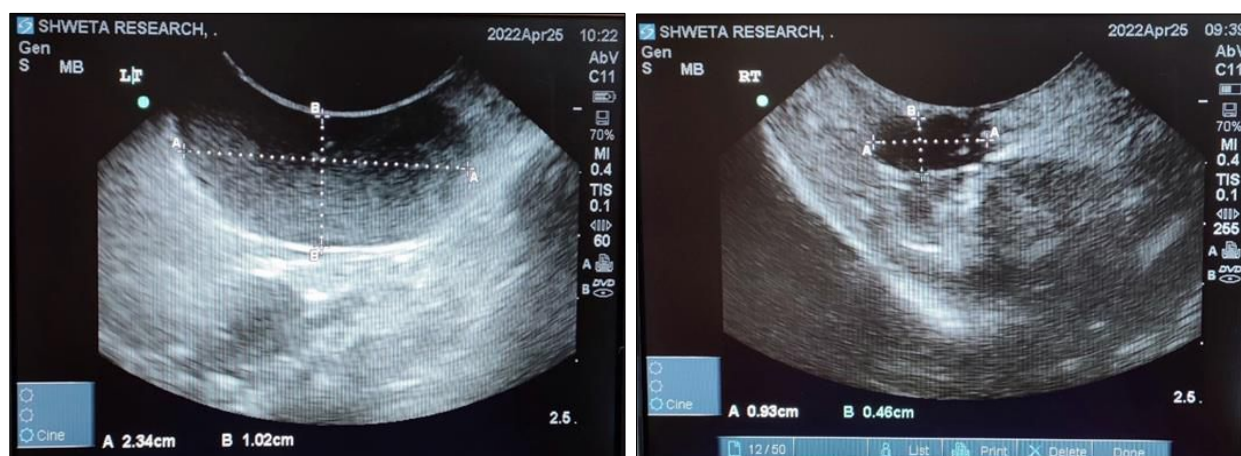


Fig 4.21: Control group. Echogenicity of testicles on day 30 (Grade 3), **Fig 4.22:** Group T₁. Echogenicity of testicles on day 30 (Grade 1)



Fig 4.23: Group T₂. Echogenicity of testicles on day 30 (Grade 1)

Haematology

No significant difference was observed in TEC, HB, PCV, Platelet count within the group and between the group. There was a significant there was no significant difference in the values of total leucocyte count on day 0, day 10 and day 30 in control group. But in group T₁ and T₂ highly

significant ($P < 0.01$) difference in the value of total leucocyte count on day 0, day 10 and day 30 was observed. Total leucocyte count on day 0 was within normal limit but on day 10 highly significant ($P < 0.01$) increase in the total leukocyte count was observed which then reached to normal value on day 30.

Table 4.8: Effect of intratesticular injection of calcium chloride on Haematological parameters (Mean $\pm$ SE) on day 0, 10 and 30 in male rats

Group/Parameter		Control (n=6)	Treatment group T ₁ (n=6)	Treatment group T ₂ (n=6)	P value
TEC (10 ⁶ /μl)	0 day	8.33 $\pm$ 0.16	8.25 $\pm$ 0.51	7.63 $\pm$ 0.28	0.328
	10 day	7.97 $\pm$ 0.22	8.30 $\pm$ 0.43	8.00 $\pm$ 0.21	0.701
	30 day	7.95 $\pm$ 0.17	7.60 $\pm$ 0.57	7.48 $\pm$ 0.26	0.663
	P value	0.290	0.564	0.351	
Hb (gm/dl)	0 day	16.02 $\pm$ 0.36	16.13 $\pm$ 0.69	16.03 $\pm$ 0.14	0.981
	10 day	16.05 $\pm$ 0.17	15.72 $\pm$ 0.41	15.61 $\pm$ 0.29	0.598
	30 day	15.73 $\pm$ 0.19	15.43 $\pm$ 0.86	15.10 $\pm$ 0.42	0.733
	P value	0.644	0.768	0.136	
PCV (%)	0 day	43.63 $\pm$ 1.61	43.90 $\pm$ 1.92	42.53 $\pm$ 0.53 _A	0.79
	10 day	42.73 $\pm$ 0.64	42.78 $\pm$ 1.84	43.00 $\pm$ 0.56 _A	0.985
	30 day	41.36 $\pm$ 1.22	37.98 $\pm$ 2.04	37.91 $\pm$ 2.21 _B	0.357
	P value	0.441	0.105	0.033*	
Platelets (10 ³ / μl)	0 day	774.0 $\pm$ 104.25	625.3 $\pm$ 52.98	661.8 $\pm$ 21.92	0.309
	10 day	669.0 $\pm$ 18.96	646.3 $\pm$ 35.20	608.0 $\pm$ 42.21	0.45
	20 day	744.17 $\pm$ 87.36	804.0 $\pm$ 103.6	704.8 $\pm$ 48.47	0.703
	P value	0.637	0.179	0.248	
TLC (10 ³ / μl)	Day 0	6.02 $\pm$ 0.48	6.00 $\pm$ 0.76 _B	4.42 $\pm$ 0.30 _C	0.091
	Day 10	5.85 $\pm$ 0.49 ^c	19.13 $\pm$ 0.51 ^a _A	15.75 $\pm$ 1.02 ^b _A	0.001**
	Day 30	7.05 $\pm$ 0.58	7.55 $\pm$ 0.61 _B	8.05 $\pm$ 0.32 _B	0.416
	P value	0.237	0.001**	0.001**	
Neutrophil (10 ³ / μl)	Day 0	1.00 $\pm$ 0.04	1.17 $\pm$ 0.20 _B	0.82 $\pm$ 0.07 _B	0.182
	Day 10	1.12 $\pm$ 0.79 ^c	8.31 $\pm$ 0.28 ^a _A	5.83 $\pm$ 0.67 ^b _A	0.001**
	Day 30	1.32 $\pm$ 0.47	1.41 $\pm$ 0.29 _B	1.83 $\pm$ 0.38 _B	0.352
	P value	0.216	0.001**	0.001**	
Lymphocyte (10 ³ / μl)	Day 0	4.63 $\pm$ 0.44	4.38 $\pm$ 0.49 _B	3.28 $\pm$ 0.22 _C	0.071
	Day 10	4.32 $\pm$ 0.43 ^b	9.56 $\pm$ 0.43 ^a _A	8.83 $\pm$ 0.46 ^a _A	0.001**
	Day 30	5.31 $\pm$ 0.47	5.65 $\pm$ 0.46 _B	5.71 $\pm$ 0.42 _B	0.965
	P value	0.300	0.001**	0.001**	
Monocyte (10 ³ / μl)	Day 0	0.33 $\pm$ 0.03	0.40 $\pm$ 0.07 _B	0.22 $\pm$ 0.04 _C	0.069
	Day 10	0.32 $\pm$ 0.04 ^c	1.03 $\pm$ 0.06 ^a _A	0.87 $\pm$ 0.06 ^b _A	0.001**
	Day 30	0.37 $\pm$ 0.05	0.38 $\pm$ 0.05 _B	0.37 $\pm$ 0.05 _B	0.391
	P value	0.693	0.001**	0.001**	
Eosinophil (10 ³ / μl)	Day 0	0.08 $\pm$ 0.02	0.12 $\pm$ 0.03 _B	0.05 $\pm$ 0.02 _B	0.179
	Day 10	0.08 $\pm$ 0.02 ^b	0.23 $\pm$ 0.03 ^a _A	0.25 $\pm$ 0.04 ^a _A	0.005**
	Day 30	0.10 $\pm$ 0.06	0.10 $\pm$ 0.00 _B	0.13 $\pm$ 0.02 _B	0.799
	P value	0.799	0.005**	0.001**	
Basophil (10 ³ / μl)	Day 0	0.00 $\pm$ 0.00	0.02 $\pm$ 0.02	0.00 $\pm$ 0.00	0.391
	Day 10	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	
	Day 30	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	
	P value		0.391		

Note: The mean bearing different superscripts within the rows and subscripts in column differ significantly. $P < 0.05$ *Significant, $P < 0.01$ **highly significant

4.4.2 Biochemical Estimation

4.4.2.1 Serum Aspartate aminotransferase (AST) level

The mean of the serum AST level of three groups on day 0, day 10 and day 30 is presented in Table 4.9.

Table 4.9: Effect of intratesticular injection of calcium chloride on serum AST level (Mean $\pm$ SE) (U/L) on day 0, 10 and 30 in male rats

Group/Period	Control group (n=6)	Treatment group T ₁ (n=6)	Treatment group T ₂ (n=6)	P value
Day 0	222.61 $\pm$ 16.34	197.24 $\pm$ 15.16	179.85 $\pm$ 14.49	0.175
Day 10	196.83 $\pm$ 19.05	198.24 $\pm$ 15.59	187.21 $\pm$ 19.29	0.896
Day 30	170.39 $\pm$ 15.53	205.17 $\pm$ 16.34	155.62 $\pm$ 15.99	0.112
P value	0.13	0.927	0.399	

The mean serum AST level in control group on day 0, day 10 and day 30 showed no significant difference, similarly in group T₁ and T₂ on day 0, day 10 and day 30 no significant difference was recorded. The mean serum AST level on day 0, day 10 and day 30 did not differ significantly between the groups.

Serum Malondialdehyde level

The mean of the serum MDA level of three groups on day 0, day 10 and day 30 is presented in Table 4.10 and Fig.4.30.

Table 4.10: Effect of intratesticular injection of calcium chloride on serum MDA level (Mean $\pm$ SE) (nmol/ml) on day 0, 10 and 30 in male rats

Group/Period	Control group (n=6)	Treatment group T ₁ (n=6)	Treatment group T ₂ (n=6)	P value
Day 0	0.83 $\pm$ 0.10 _B	0.81 $\pm$ 0.10 _B	0.63 $\pm$ 0.07 _B	0.27
Day 10	1.18 $\pm$ 0.13 _A	1.80 $\pm$ 0.19 _A	1.71 $\pm$ 0.23 _A	0.078
Day 30	0.55 $\pm$ 0.11 _B	0.86 $\pm$ 0.09 _B	0.58 $\pm$ 0.10 _B	0.97
P value	0.005**	0.001**	0.001**	

Note: The mean bearing different superscripts within the rows and subscripts in column differ significantly. $P < 0.01$ **highly significant

The mean serum MDA level in control, T₁ and T₂ group showed significant difference, value on day 0 differed highly significantly ($P < 0.01$) from day 10 but not with value on day 30 and MDA level on day 10 also differed significantly from day 30 level. The mean serum MDA level on day 0, day 10 and day 30 did not differ significantly between the group. But, serum MDA level on day 10 shows the non-significant increase in T₁ and T₂ group as compared to control group. This elevation in serum MDA level on day 10 may be due to the damage caused by intratesticular injection leading to lipid peroxidation of the cell membrane.

Discussion: The swelling of the testis as seen in the present study was in accordance with the findings of Jana *et al.* (2002); Jana and Samantha (2006); Maadi *et al.* (2021) in rats, Jana and Samantha (2007); Oliveira *et al.* (2012a); Howaida and Abu-Ahmed (2015); Canpolat *et al.* (2016); Puri *et al.* (2018); Thakre *et al.* (2021) in dogs, Jana *et al.* (2005), Hasan *et al.* (2020) in bucks, Jana and Samantha (2011) in tom cats and Sen *et al.* (2017) in guinea pigs following the injection of different sterilizing agents. While study carried out for chemosterilization by Canpolat *et al.* (2006b) in bulls, De Brito *et al.* (2020) in rats, Leoci *et al.* (2014a); Leoci *et al.* (2014b); Canpolat *et al.* (2006a); Silva *et al.* (2018); Leoci *et al.* (2019); Ali *et al.* (2020) [1] in dogs there were no similar findings as of this study were recorded. De Macedo *et al.* (2018) used the dipyrone + meloxicam following the intratesticular injection of Zinc gluconate and opined that there was no suppression of local inflammatory response in rats. This supports the present study. They have used various agents like dipyrone, meloxicam, celecoxib and dexamethasone to control the local reactions following intratesticular injection. But none was found effective to control the inflammatory for analgesic effect following intratesticular injection of zinc gluconate. Similarly, Oliveira *et al.* (2012b) also did not find beneficial effects of dipyrone (anti-inflammatory, analgesic and antipyretic) following intra-testicular injection of Zinc gluconate in dogs [6, 13, 14, 23, 26, 29-33, 40-42, 43, 45, 46, 48, 53, 58].

Jana *et al.* (2002) found an elevation in the rectal temperature in rats by 3rd day after intratesticular injection of calcium chloride, which supports the findings of the present study, Jana and Samantha (2006); Maadi *et al.* (2021) in rats, Jana and Samantha (2007); Oliveira *et al.* (2012a); Howaida and Abu-Ahmed (2015); Canpolat *et al.* (2016); Puri *et al.* (2018); Thakre *et al.* (2021) in dogs, Jana *et al.* (2005), Hasan *et al.* (2020) in bucks, Jana and

Samantha (2011) in tom cats and Sen *et al.* (2017) in guinea pigs such findings were not observed. In rats of the T₂ group, rectal temperature was elevated only on the next day after intratesticular injection and not on the following days. This may be attributed to the effect of the meloxicam injection given subcutaneously as meloxicam is having an antipyretic effect [6, 23, 26, 29-33, 43, 45, 48, 51, 58].

Similar results to those of the current study were found in rats by Jana and Samanta (2006), Smita (2011), Das and Karmakar (2016), Karmakar and Das (2017), and De Brito *et al.* (2020); no significant variation in body weight was seen after intratesticular injection of a different sterilizing agent. Fesseha and Negash (2020) in rats and Sen *et al.* (2017) in guinea pigs both found a significant increase in body weight in the treatment group animals following intratesticular injection of different chemo-sterilant which contradict the present work [12, 13, 18, 35, 36, 51, 54].

Alterations in echogenicity over the time were recorded where it first increased and then the gradual decrease in echogenicity. Similar trends of variation in echogenicity were observed by Canpolat *et al.* (2006b) in bulls, Canpolat *et al.* (2006a); Canpolat *et al.* (2016), Rafatmah *et al.*, (2019) in dogs; Laku *et al.* (2021) in bucks following the intratesticular injection of various chemical agents. Hami *et al.* (2020) in rats, Cicirelli *et al.* (2022) in dogs, found reduction in the echogenicity but no acute increase in echogenicity was observed by them [4-6, 9, 22, 39, 49].

Total leucocyte count increase was also observed by Smita (2011) following intratesticular injection of sterilizing agent in rats. While no similar findings was observed by Kadam (2014) and De Brito *et al.* (2020) following intratesticular injection of sterilizing agent in rats [13].

Serum AST level observed in the present study was found to be in agreement with the observations of Smita (2011); Kadam (2014); Das and Karmakar (2016) and Karmakar and Das (2017) in rats, Chatterjee *et al.* (2009) and Oliveira *et al.* (2012a) in dogs following intratesticular injection of either calcium chloride or another sterilizing agent.

Jana *et al.* (2002); Jana *et al.* (2005); Jana and Samanta (2006); Chatterjee *et al.* (2009); Jana and Samanta (2011) found elevation in the MDA content in testicular tissue following intratesticular injection [7, 12, 29-33, 34, 35, 36, 44, 54].

References

1. Ali M, Ijaz M, Manzoor A, Mohy-Ud-Din MT, Hassan F, Tabassum R, *et al.* Efficacy of *Azadirachta indica* (Neem) leaf extract and hypertonic saline solution as intratesticular chemical sterilizing agents in dogs. Braz

- J Vet Res Anim Sci. 2020;57(4):e171582. doi:10.11606/issn.1678-4456.bjvras.2020.171582.
2. Ansari AS, Badar A, Lohiya NK. Fertility control modalities in animals: an overview. BAOJ Vet Sci. 2017;1:004.
3. Bakir B, Gulyuz F, Karaca F, Yuksel H, Sahin A, Uslu BA. Chemical castration in dogs. J Health Sci. 2002;8(1-2):6-9.
4. Canpolat I, Kilic S, Cevik A. Chemosterilization of male dogs. Indian Vet J. 2006;83(1):110-111.
5. Canpolat I, Gur S, Gunay C, Bulut S, Eroksuz H. Evaluation of bull castration by intra-testicular injection of ethanol and calcium chloride. Rev Med Vet. 2006;157(8-9):420.
6. Canpolat I, Karabulut E, Eroksuz Y. Chemical castration of adult and non-adult male dogs with sodium chloride solution. J Agric Vet Sci. 2016;9:9-11. doi:10.9790/2380-0912010911.
7. Chatterjee K, Ali KM, De D, Mallick C, Ghosh D. Induction of chemosterilization by single intratesticular calcium chloride injection in stray dogs. Res J Anim Vet Sci. 2009;4:22-29.
8. Chaudhary R, Sharma AK, Mehra US, Dutt R, Kumar P, Yadav R. Histopathological studies on intra-epididymal zinc arginine administration in pre-pubertal dogs. Int J Curr Microbiol Appl Sci. 2018;7(5):569-581. doi:10.20546/ijcmas.2018.705.071.
9. Cicirelli V, Macrì F, Di Pietro S, Leoci R, Lacalandra GM, Aiudi GG. Use of contrast-enhanced ultrasound of the testes after non-surgical sterilization of male dogs with CaCl₂ in alcohol. Animals. 2022;12(5):577. doi:10.3390/ani12050577.
10. Cohen RDH, King BD, Thomas LR, Janzen ED. Efficacy and stress of chemical versus surgical castration of cattle. Can J Anim Sci. 1990;70(4):1063-1072. doi:10.4141/cjas90-129.
11. Das RP, Mustafa AS, Talwar GP. Atrophy of seminiferous tubules of mouse testes after intratesticular injection of BCG and their regeneration. Arch Androl. 1982;9(3):245-250. doi:10.3109/01485018208990246.
12. Das SK, Karmakar SN. Potential effects of calcium chloride as a chemical castrative agent on male albino rat. Int J Appl Biol Pharm Technol. 2016;7(3):114-120. doi:10.21276/ijabpt.2016.7.3.14.
13. De Brito JLM, Lima VND, Ansa DO, Moya SE, Morais PC, Azevedo RBD, *et al.* Acute reproductive toxicology after intratesticular injection of silver nanoparticles in Wistar rats. Nanotoxicology. 2020;14(7):893-907. doi:10.1080/17435390.2020.1774812.
14. De Macedo SRB, De Lima LAR, De Torres SM, De Oliveira VVG, De Morais RN, Peixoto CA, *et al.* Effects of intratesticular injection of zinc-based solution in rats combined with anti-inflammatory and analgesic drugs during chemical sterilization. Vet World. 2018;11(5):649-656. doi:10.14202/vetworld.2018.649-656.
15. Dixit VP, Lohiya NK, Arya M, Agrawal M. Chemical sterilization of male dogs after a single intra-testicular injection of danazol. Folia Biol. 1975;23(3):305-310.
16. Fahim MS, Wang M, Sutcu MF, Fahim Z, Youngquist RS. Sterilization of dogs with intra-epididymal injection of zinc arginine. Contraception. 1993;47(1):107-122. doi:10.1016/0010-7824(93)90113-L.
17. Feher T, Bodrogi L, Makray S. Dynamics of serum testosterone levels in bulls daily, seasonally and after chemical or surgical castration. Magy Allatorv Lapja. 1985;40:13-16.
18. Fesseha H, Negash G. Evaluation of single bilateral intratesticular injection of cetrimide for nonsurgical sterilization of adult male albino mice. Insights Vet Sci. 2020;4(1):25-34. doi:10.29328/journal.ivs.1001023.
19. Fordyce G, Beaman NJ, Laing AR, Hodge PB, Campero C, Shepherd RK. Evaluation of calf castration by intra-testicular injection of a lactic acid solution. Aust Vet J. 1989;66(9):272-276. doi:10.1111/j.1751-0813.1989.tb13950.x.
20. Giri SC, Yadav BPS, Panda SK. Chemical castration in pigs. Indian J Anim Sci. 2002;72(6):451-453.
21. Gunay C, Sonmez M, Turk G. Comparison of the effects of intratesticular glycerol and ethanol injections on sterilization of male dogs. Turk J Vet Surg. 2004;10:55-60.
22. Hami M, Veshkini A, Jahandideh A, Rafiee SM, Mortazavi P. Ultrasonographic and histopathologic study of chemical castration with calcium chloride solution in rat testis. J Crit Rev. 2020;7(1). doi:10.22159/jcr.07.01.01.
23. Hasan M, Miah MAH, Alam MM, Juyena NS. Clinical evaluation of chemo-sterilization through histomorphology and hormonal changes in bucks. S Afr J Anim Sci. 2020;50(6). doi:10.4314/sajas.v50i6.12.
24. Heath E, Arowolo R. Early histopathologic effects of intratesticular injection with hyperosmolar glycerol, glucose or NaCl solutions. Andrologia. 1987;19(6):654-661. doi:10.1111/j.1439-0272.1987.tb01922.x.
25. Hill GM, Neville WE Jr, Richardson KL, Utley PR, Stewart RL. Castration method and progesterone-estradiol implant effects on growth rate of suckling calves. J Dairy Sci. 1985;68(11):3059-3061. doi:10.3168/jds.S0022-0302(85)81202-9.
26. Howaida M, Abu-Ahmed. Chemical sterilization of dogs using single bilateral intratesticular injection of calcium chloride or clove oil. Alex J Vet Sci. 2015;45(1):26-32. doi:10.5455/ajvs.179468.
27. Ijaz A, Abalkhail AA, Khamas WAH. Effect of intratesticular injection of formalin on seminiferous tubules in Awassi lambs. Pak Vet J. 2000;20(3):129-134.
28. Immegart HM, Threlfall WR. Evaluation of intratesticular injection of glycerol for nonsurgical sterilization of dogs. Am J Vet Res. 2000;61(5):544-549. doi:10.2460/ajvr.2000.61.544.
29. Jana K, Samanta PK. Evaluation of single intratesticular injection of calcium chloride for nonsurgical sterilization in adult albino rats. Contraception. 2006;73(3):289-300. doi:10.1016/j.contraception.2005.07.011.
30. Jana K, Samanta PK. Sterilization of male stray dogs with a single intratesticular injection of calcium chloride: a dose-dependent study. Contraception. 2007;75(5):390-400. doi:10.1016/j.contraception.2007.01.022.
31. Jana K, Samanta PK. Clinical evaluation of non-surgical sterilization of male cats with single intratesticular injection of calcium chloride. BMC Vet Res. 2011;7:39. doi:10.1186/1746-6148-7-39.
32. Jana K, Samanta PK, Ghosh D. Dose-dependent response to an intratesticular injection of calcium chloride for induction of chemosterilization in adult

- albino rats. *Vet Res Commun*. 2002;26(8):651-673. doi:10.1023/A:1020976905746.
33. Jana K, Samanta PK, Ghosh D. Evaluation of single intratesticular injection of calcium chloride for nonsurgical sterilization of male Black Bengal goats (*Capra hircus*): a dose-dependent study. *Anim Reprod Sci*. 2005;86(1-2):89-108. doi:10.1016/j.anireprosci.2004.05.021.
 34. Kadam DP. Effect of single intratesticular injection of cadmium chloride in Wistar rat for chemical sterilization [PhD thesis]. Mumbai: Bombay Veterinary College, Maharashtra Animal and Fishery Sciences University; 2014.
 35. Kar AB, Kamboj VP, Goswami A. Sterilization of male rhesus monkeys by iron salts. *J Reprod Fertil*. 1965;9(1):115-117. doi:10.1530/jrf.0.0090115.
 36. Karmakar SN, Das SK. Chemosterilization induced by intratesticular injection of calcium chloride (CaCl₂): a tool for population control. *Int J Pharm Chem Biol Sci*. 2017;7(1):25-35.
 37. Koger L. Calcium chloride castration. *Mod Vet Pract*. 1978;59(2):119-121.
 38. Kutzler M, Wood A. Non-surgical methods of contraception and sterilization. *Theriogenology*. 2006;66(3):514-525. doi:10.1016/j.theriogenology.2006.04.014.
 39. Laku D, Mohammed A, Bukar MM. Effectiveness of castration with Burdizzo, calcium chloride and olive oil in Sahel bucks. *Sahel J Vet Sci*. 2021;18(1):13-20. doi:10.54058/saheljvs.v18i1.213.
 40. Leoci R. Calcium chloride dehydrate nonsurgical sterilization in 81 dogs: dose, formulation and best practices implications for maximal effectiveness and minimal complications. In: *Proceedings of the 1st International Conference on Dog Population Management*; 2012.
 41. Leoci R, Aiudi G, Cicirelli V, Brent L, Iaria C, Lacalandra GM. Effects of intratesticular vs intraepididymal calcium chloride sterilant on testicular morphology and fertility in dogs. *Theriogenology*. 2019;127:153-160. doi:10.1016/j.theriogenology.2019.01.006.
 42. Leoci R, Aiudi G, Silvestre F, Lissner EA, Lacalandra GM. Alcohol diluent provides the optimal formulation for calcium chloride non-surgical sterilization in dogs. *Acta Vet Scand*. 2014;56:62. doi:10.1186/s13028-014-0062-2.
 43. Maadi MA, Behfar M, Rasaei A, Shalazar-Jalali A, Najafi G, Mohammadi V. Chemical castration using an intratesticular injection of mannitol: a preliminary study in a rat model. *Turk J Vet Anim Sci*. 2021;45(3):519-530. doi:10.3906/vet-2010-111.
 44. Oliveira EC, Moura MR, Silva VA Jr, Peixoto CA, Saraiva KL, De Sá MJC, *et al*. Intratesticular injection of a zinc-based solution as a contraceptive for dogs. *Theriogenology*. 2007;68(2):137-145. doi:10.1016/j.theriogenology.2007.03.026.
 45. Oliveira EC, Moura MRP, de Sá MJ, Silva VA Jr, Kastelic JP, Douglas RH, *et al*. Permanent contraception of dogs induced with intratesticular injection of a zinc gluconate-based solution. *Theriogenology*. 2012;77(6):1056-1063. doi:10.1016/j.theriogenology.2011.10.008.
 46. Oliveira EC, Muller PM, Silva FL, Nery LT, de Sá MJ, Guerra MMP, *et al*. Oral administration of an anti-inflammatory does not compromise the efficacy of intratesticular injection of zinc gluconate as a contraceptive for dogs. *Anim Reprod Sci*. 2012;132(3-4):207-212. doi:10.1016/j.anireprosci.2012.05.003.
 47. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother*. 2010;1(2):87. doi:10.4103/0976-500X.72350.
 48. Puri B, Shah MK, Thakur B, Regmi B, Dhakal I. Intratesticular injection of calcium chloride is a useful alternative for neutering the male dog. *Int J Appl Sci Biotechnol*. 2018;6(2):158-163. doi:10.3126/ijasbt.v6i2.20431.
 49. Rafatmah D, Mogheiseh A, Eshghi D. Chemical sterilization with intratesticular administration of zinc gluconate in adult dogs: a preliminary report. *Basic Clin Androl*. 2019;29:9. doi:10.1186/s12610-019-0092-8.
 50. Russell LD, Saxena NK, Weber JE. Intratesticular injection as a method to assess the potential toxicity of various agents and to study mechanisms of normal spermatogenesis. *Gamete Res*. 1987;17(1):43-56. doi:10.1002/mrd.1120170106.
 51. Sen CC, Yumusak N, Faundez R, Temamogullari F, Taskin A. Evaluation of intra-testicular injections of calcium chloride and 4-vinylcyclohexene monoepoxide for chemical sterilization in guinea pigs. *Pol J Vet Sci*. 2017;(2). doi:10.1515/pjvs-2017-0030.
 52. Shemi D, Marx Z, Kaplanski J, Potashnik G, Sod-Moriah UA. Testicular damage development in rats injected with dibromochloropropane. *Andrologia*. 1988;20(4):331-337. doi:10.1111/j.1439-0272.1988.tb00697.x.
 53. Silva RC, Paranzini CS, Franco LG, Miguel MP, Honsho CS, Souza FF. Calcium chloride combined with dimethyl sulphoxide for the chemical sterilization of dogs. *Reprod Domest Anim*. 2018;53(6):1330-1338. doi:10.1111/rda.13252.
 54. Smita. Chemical sterilization of male Wistar rat by using cadmium chloride [MVSc thesis]. Mumbai: Bombay Veterinary College, Maharashtra Animal and Fishery Sciences University; 2011.
 55. Sprando RL, Black TN, Ames MJ, Rorie JJ, Collins TFX. Effect of intratesticular injection of sodium fluoride on spermatogenesis. *Food Chem Toxicol*. 1996;34(4):377-384. doi:10.1016/0278-6915(96)84542-X.
 56. Soerensen B, Soutello RVG, Gomes CRA, Garcia R, Marini A, Fonza JFF, *et al*. Chemical, surgical and mechanical castration of bovine. *Cienc Agrar Saude*. 2001;1(2):7-10.
 57. Taylor LH, Wallace RM, Balaram D, Lindenmayer JM, Eckery DC, Mutoonono-Watkiss B, *et al*. The role of dog population management in rabies elimination a review of current approaches and future opportunities. *Front Vet Sci*. 2017;4:109. doi:10.3389/fvets.2017.00109.
 58. Thakre T, Shukla S, Mishra A, Gupta N, Kumar P. Sterilization of male dogs by intra-testicular administration of calcium chloride solution. *Indian J Anim Res*. 2021;1:1-5.
 59. Weinbauer GF, Galhotra MM, Nieschlag E. Focal testicular destruction following intratesticular injection of glycerol in rats. *Int J Androl*. 1985;8(5):365-375. doi:10.1111/j.1365-2605.1985.tb00849.x.